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Allelopathic potential of citrus fruit peel and abscisic acid-glucose ester

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Abstract

Aqueous methanol extracts of *Citrus junos*, *C. unshiu* and *C. hassaku* fruit peel inhibited the growth of the roots and hypocotyls of lettuce (*Lactuca sativa* L.) seedlings. Significant reductions in the root and hypocotyl growth were observed as the extract concentration increased in all bioassays. The inhibitory activity of *C. junos* extract on the growth of lettuce roots and hypocotyls was about 13- and 24-fold greater than that of *C. unshiu* and *C. hassaku* extracts, respectively. The concentration of abscisic acid-β-D-glucopyranosyl ester (ABA-GE) in fruit peel of *C. junos*, *C. unshiu* and *C. hassaku* was determined since ABA-GE had been found to be a possible cause of the growth inhibitory effect of *C. junos*. The concentration was 132, 10.6 and 5.0 μg g^{-1} dry weight in *C. junos*, *C. unshiu* and *C. hassaku* fruit peel, respectively. Thus, there was a good correlation between ABA-GE concentrations in *C. junos*, *C. unshiu* and *C. hassaku* fruit peel and the inhibitory activities of their aqueous extracts, which suggests that ABA-GE may be involved in the growth inhibitory effect of *C. junos*, *C. unshiu* and *C. hassaku* fruit peel.

Abbreviations: ABA-GE – abscisic acid-β-D-glucopyranosyl ester

Introduction

The peel of *Citrus junos* fruit has been found to possess potent allelopathic activity because an aqueous methanol extract of the peel inhibited the growth of several weed species. The powder of the peel also inhibited these weed species and was useful as a weed suppressive agent (Fujihara and Shimizu 1999). Although abscisic acid-β-D-glucopyranosyl ester (ABA-GE; Figure 1) had been known to be a physiologically inactive conjugated ABA (Milborrow 1970, 1978; Neill et al. 1983; Zeevaart 1983; Lehman and Vlasov 1988), the compound causing the inhibitory effect of an aqueous methanol extract of *C. junos* fruit peel was isolated and identified as ABA-GE. Exogenously applied ABA-GE also inhibited hypocotyl and root growth of lettuce seedlings at concentrations greater than 0.3 μM (Kato-Noguchi et al. 2002). Thus, ABA-GE may play an important role in the allelopathy of *C. junos* fruit peel. However, there are few reports of allelopathic potential associated with ABA-GE concentration on other plant species. In this study the allelopathic potential of *C. junos*, *C. unshiu* and *C. hassaku* fruit peel and the concentration of ABA-GE in their peel were determined.

Materials and methods

Plant material and extraction

Freeze-dried fruit peel (5 g dry weight) of *Citrus junos* Sieb. ex Tanaka, *Citrus unshiu* Marcov and *Citrus hassaku* hort. ex. Tanaka were powdered in a mortar using a pestle and extracted with 100 ml of 80% aqueous cold methanol for three days at 4 °C. After filtration using filter paper (No. 2; Toyo, Tokyo, Ja-

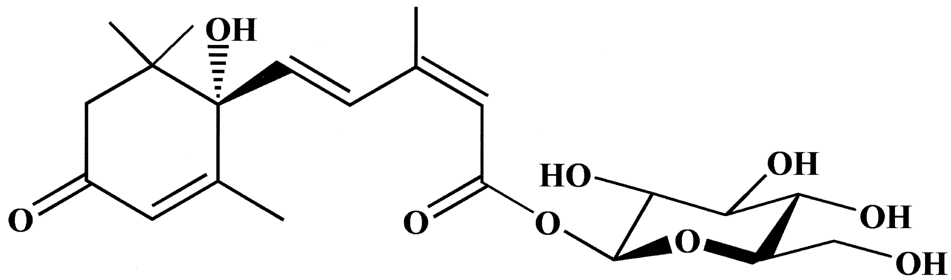

Figure 1. Chemical structure of abscisic acid-β-D-glucopyranosyl ester

pan), the filtrate was evaporated to dryness at 40 °C in vacuo.

Bioassay

Test samples were dissolved in a small volume of methanol and water, applied to a sheet of filter paper (No. 2) in a 3.5-cm Petri dish and dried. The filter paper in the Petri dish was moistened with 0.8 ml of 0.05% (v/v) Tween 20. Then, ten seeds of lettuce (*Lactuca sativa* L.) were arranged on the filter papers in the Petri dishes and allowed to grow in the dark at 25 °C for 60 h. The root and hypocotyls lengths of the seedlings were then measured with a ruler and the percentage length of seedlings was calculated by reference to the length of control plants treated with plain solution.

Quantification of ABA-GE

Freeze-dried fruit peel of *C. junos*, *C. unshiu* and *C. hassaku* was powdered and extracted with aqueous cold methanol as describe above. The extract was purified by using ion exchange columns and a reverse-phase C_{18} Sep-Pak cartridge according to the procedure described by Kato-Noguchi et al. (2002). Then, the sample of ABA-GE was injected onto a HPLC column (4.6 mm i.d. × 15 cm, Hydrosphere C_{18}; YMC Ltd, Kyoto, Japan) which was eluted at a flow rate of 0.8 ml min^{-1} with 22% aqueous methanol; detected at 280 nm. Quantification of ABA-GE was preformed by interpolating the peak height on the chromatograms of HPLC to a standard curve constructed by the peak height of pure ABA-GE isolated from peel of *C. junos* fruit (Kato-Noguchi et al. 2002). The overall recoveries of ABA-GE through the entire quantification process for *C. junos*, *C. unshiu* and *C. hassaku* were 73–78%.

Results and discussion

Allelopathic potential of citrus fruit peel

The allelopathic potential of aqueous methanol extracts of *C. junos*, *C. unshiu* and *C. hassaku* fruit peel was tested by measuring root and hypocotyl growth of lettuce seedlings (Figure 2). The root growth of lettuce seedlings was inhibited at concentrations greater than 0.1, 3.0 and 3.0 mg dry weight equivalent extract ml^{-1} for *C. junos*, *C. unshiu* and *C. hassaku*, respectively. The hypocotyl growth of lettuce seedlings was inhibited at concentrations greater than 0.3, 3.0 and 3.0 mg dry weight equivalent extract ml^{-1} for *C. junos*, *C. unshiu* and *C. hassaku*, respectively. Increasing the concentrations increased the inhibition of both root and hypocotyl growth in all bioassays. When the percentage length was plotted against the logarithm of the concentrations, the response curves for roots and hypocotyls were linear between 20–80% inhibition. As interpolated from the concentration response curves, the concentrations required for 50% inhibition of root growth in the assay (defined I_{50}) were 0.37, 4.6 and 8.8 mg dry weight equivalent extract ml^{-1} for *C. junos*, *C. unshiu* and *C. hassaku*, respectively, and of hypocotyls growth in the assay were 0.55, 7.6 and 13 mg dry weight equivalent extract ml^{-1} for *C. junos*, *C. unshiu* and *C. hassaku*, respectively. Comparing I_{50} values, the inhibitory activities of *C. junos* extract on the root and hypocotyl growth were about 13- and 24-fold greater than those of *C. unshiu* and *C. hassaku* extracts, respectively. These results suggest that the aqueous methanol extracts of *C. junos*, *C. unshiu* and *C. hassaku* possesses allelopathic potential and *C. junos* extract has the greatest activity.

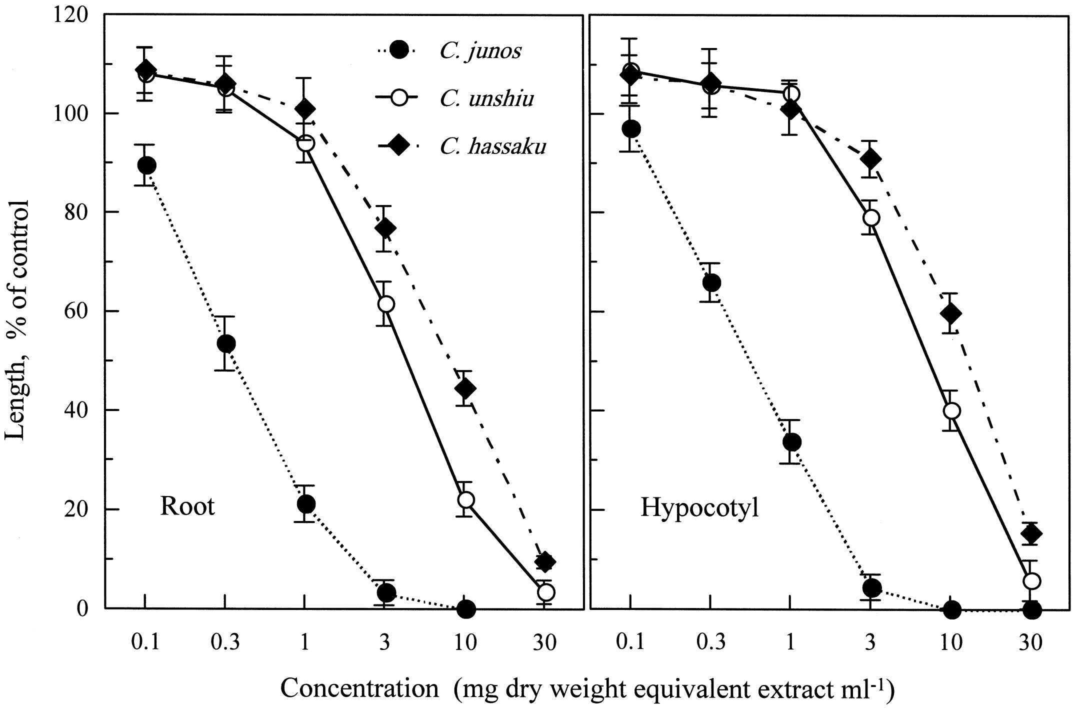

Figure 2. Effects of aqueous methanol extracts of *C. junos*, *C. unshiu* and *C. hassaku* fruit peel on the growth of roots and hypocotyls of lettuce seedlings. Root and hypocotyl length was determined after 60 h of incubation in the dark at 25 °C. Means ± SE from three independent experiments with 10 plants for each determination are shown. Root and hypocotyl length of control plants were 16.8. ± 1.2 mm and 6.7 ± 0.5 mm, respectively.

Concentration of ABA-GE

The concentration of ABA-GE in *C. junos*, *C. unshiu* and *C. hassaku* fruit peel was determined since the main growth-inhibiting substance causing the inhibitory effect of *C. junos* fruit peel was identified as ABA-GE (Figure 1) although *C. junos* fruit peel may contain other growth inhibitors (Kato-Noguchi et al. 2002). Figure 3 shows that the concentration in *C. junos* fruit peel was the greatest being 13- and 26-fold greater than that in *C. unshiu* and *C. hassaku*, respectively. Thus, the concentrations of ABA-GE in the fruit peel of *C. junos*, *C. unshiu* and *C. hassaku* and the inhibitory activities of their aqueous methanol extracts show a good correlation (Figures 2 and 3). These findings together with the effectiveness of ABA-GE on growth inhibition (Kato-Noguchi et al. 2002) suggest that ABA-GE may be involved in the allelopathy of citrus fruit peel.

ABA-GE accumulates in plant tissues with age and during stress treatments, and was known to be a physiologically inactive conjugated ABA and an end-product of ABA metabolism rather than a storage or transport form (Milborrow 1970, 1978; Neill et al. 1983; Zeevaart 1983; Lehman and Vlasov 1988). However, recently an ABA-GE-cleaving enzyme, apoplastic β-D-glucosidase, which releases free ABA

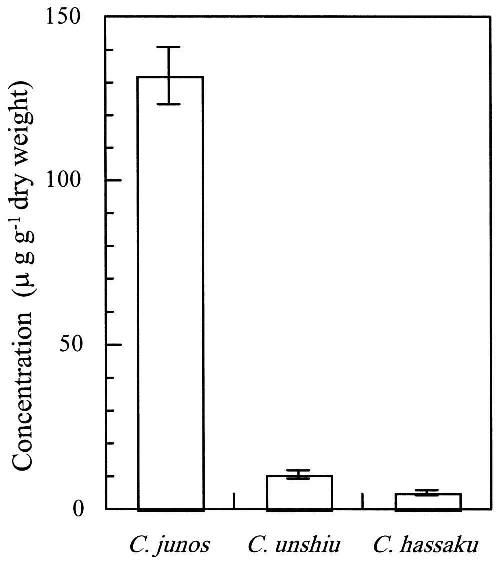

Figure 3. Concentration of ABA-GE in *C. junos*, *C. unshiu* and *C. hassaku* fruit peel. Means ± SE from three independent experiments with three assays for each determination are shown.

from the ABA-GE conjugate pool in the plant tissues, was found in maize and barley (Dietz et al. 2000; Sauter and Hartung 2000). It also has been shown that ABA-GE can be translocated in the xylem sap from roots to shoots of several plants (Bano et al. 1994; Jeschke et al. 1997; Hansen and Dörffling 1999; Sauter and Hartung 2000). Furthermore, exogenous applied ABA-GE inhibited the growth of hypocotyls and roots of lettuce seedlings (Kato-Noguchi et al. 2002). Thus, ABA-GE may have important physiological functions in plants. The results presented in this paper support this hypothesis.

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